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Genotoxic and cytotoxic effects of the formulated insecticide Aficida® on *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces: Poeciliidae)

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ABSTRACT

The acute toxicity, genotoxicity, and cytotoxicity of the pirimicarb-containing commercial-formulation carbamate insecticide Aficida® (50% pirimicarb) were evaluated on *Cnesterodon decemmaculatus* (Pisces, Poeciliidae) exposed under laboratory conditions. Micronucleus (MN) induction as well as alterations in the erythrocytes:erythroblasts ratios were employed as end-points for genotoxicity and cytotoxicity, respectively. Cr(VI) and cyclophosphamide were used as positive controls for the toxicity and genocytotoxicity assays, respectively. Mean values of 344.3 and 225.5 mg Aficida®/L were determined for LC-50_{24h} and LC-50_{96h}, respectively. In 48 h-exposed fish, a MN increase was found in Aficida®-treated fish in the 50–157 mg/L concentration range. When fish were exposed to Aficida® for 96 h, only those animals treated at 50–100 mg/L showed an increase in MN frequency. Cellular cytotoxicity, revealed by a decreased proportion of circulating erythrocytes and an enhancement of erythroblasts, was found after 48 h of exposure in 50–157 mg Aficida®/L-treated fish, while, after 96 h exposure, only 100–157 mg Aficida®/L induced the same effect. This species provides a useful experimental model for the biomonitoring of aquatic ecosystems.

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1. Introduction

Large amounts of chemicals are released into the environment, worldwide, especially on croplands and pastures, with the aim of increasing agricultural production. Agrochemicals may be hazardous to the environment, because of their persistence, bioaccumulation, and toxicity (www.epa.gov/pesticides/). Pesticides affect not only target organisms but also non-target organisms [1–3]. Unfortunately, it is difficult to decrease the use of pesticides without reducing crop yields [4,5].

Agrochemical contamination of food, water, and air has become a severe problem for human and ecosystem health [6]. In agriculture, pesticides are generally not used as a single active ingredient but rather as part of complex commercial formulations. In addition to the active component, the formulated products contain solvents and adjuvants, some of which may induce damage in mammalian and insect cells [7–12]. Hence, pesticide risk assessment must also consider toxic effects caused by the excipient(s). Among the carbamate pesticides, pirimicarb is a selective insecticide used mostly for aphid control. Its mode of action is inhibition of acetylcholinesterase activity [13]. Available information indicates that 23 formulated products containing pir-

imicarb as an active ingredient have been registered worldwide (www.environmentalchemistry.com). Some of these pirimicarb-containing formulations have been reported to induce toxic effects in the microbial community of freshwater sediments [14,15], in the crustaceans *Ceriodaphnia quadrangula* [16] and *Daphnia magna* [17,18], in insects such as *Culex quinquefasciatus* [19], in fishes such as *Poecilia reticulata* and *Cyprinus carpio* [20], and in anuran tadpoles of both *Pelophylax perezii* [21,22] and *Rhinella arenarum* [23].

Genotoxic studies of pirimicarb are scarce [24,25]. Pirimicarb has been generally recognized as non-genotoxic in bacteria, yeast, fungi, and mammalian cells [26,27]. It has been reported to be non-mutagenic in *Salmonella typhimurium* with S9 metabolic activation [26], but a positive response was observed in mouse lymphoma L5178Y cells [24]. Positive results have also been reported in the w/w⁺ eye mosaic system with *Drosophila melanogaster* Oregon-K strain [28]. DNA single-strand breaks were detected by the comet assay in human lymphocytes *in vitro* [29]. Pirimicarb did not induce chromosomal alterations either in rat bone marrow cells, after oral administration [30], or in human lymphocytes *in vitro*, with or without metabolic activation [26]. However, Pilinskaia [31] observed an increased frequency of chromosomal aberrations in peripheral lymphocytes from occupationally exposed workers. An increase in the frequency of chromosomal aberrations and the induction of sister chromatid exchanges have been reported in pirimicarb-treated CHO-K1 cells [12]. Finally, we recently demonstrated the ability of its formulation Aficida® to increase the frequency of micronucleated erythrocytes on *R. arenarum* tadpoles exposed

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under laboratory conditions and to induce cytotoxicity in peripheral circulating blood cells [23].

Cnesterodon decemmaculatus is an endemic member of the fish family Poeciliidae with extensive distribution in neotropical America. The species attains high densities in a large variety of water-bodies within the entire La Plata River and other South American basins. *C. decemmaculatus* is a small, ovoviviparous, micro-omnivorous, benthic-pelagic, non-migratory fish (maximum size, ≈ 25 and 45 mm for $\sigma\sigma$ and ♀♀ , respectively), and is often the most abundant (and sometimes the only) species present in small watercourses. The species is easy to handle and acclimate to laboratory conditions. The ranges of tolerance of *C. decemmaculatus* to many environmental parameters, e.g., temperature, salinity and pH, match the conditions for many toxicity tests [32]. Furthermore, several reports found this species to be suitable as a test organism in acute and chronic toxicity bioassays [33–38].

The aim of the present study was to characterize the acute toxicity of the Argentinean pirimicarb-containing commercial-formulation carbamate insecticide Aficida® (50% pirimicarb) on *C. decemmaculatus* (Pisces, Poeciliidae) exposed under laboratory conditions, by determining mortality and biomarkers of geno- and cytotoxicity: MN in mature erythrocytes and circulating erythrocyte:erythroblast ratios, respectively. This commercial formulation was chosen because it represents one of the carbamate-containing formulations most widely used for cereal production and garden insect control in Argentina and worldwide.

2. Materials and methods

2.1. Chemicals

Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate; commercial grade, trade name Aficida®, CAS 23103-98-2; 50% pirimicarb, excipients q.s.) was kindly provided by Syngenta Agro S.A. (Buenos Aires, Argentina). Cyclophosphamide (CAS 6055-19-2) was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and $\text{K}_2\text{Cr}_2\text{O}_7$ (CAS 7778-50-9) was obtained from Merck KGaA (Darmstadt, Germany).

2.2. Quality control

Chemical analysis of pirimicarb in the test solutions was carried out using high-performance liquid chromatography (Agilent 1100) with UV diode array detection at a wavelength of 230 nm. The C_{18} column was 15 cm length, 4.6 mm inner diameter, and the mobile phase was acetonitrile in 10 mM KH_2PO_4 pH 4 buffer (60:40); flow rate was 0.8 mL/min. Pirimicarb was identified by comparison of chromatographic peaks corresponding to solutions from the initial time and 24 h after treatment, as reported elsewhere [23].

2.3. Test organisms

C. decemmaculatus was selected as the target organism. Specimens were collected from a permanent pond, free from pluvial runoff from agricultural areas, in the vicinity of La Plata city (Buenos Aires Province, Argentina). Adults were transported to the laboratory and then acclimatized for at least 20 days to 16:8 h light/dark cycles in aquaria at $20 \pm 1^\circ\text{C}$ with dechlorinated tap water (pH 7.6–8.3, hardness 250 mg/L CaCO_3) with artificial aeration, and fed *ad libitum* daily with commercially available fish food (TetraMin®, TetraWerke, Germany) until the beginning of the experimental procedures. Average body weight of the specimens was 0.26 ± 0.1 g while the mean body length was 29.5 ± 2.7 mm.

2.4. Determination of LC-50

Experiments were carried out for toxicity assessment following recommendations proposed by the USEPA standardized methods for acute toxicity testing [27,39]. Briefly, for each experimental point, ten specimens were maintained in a 1 L glass container, and exposed to ten different concentrations of Aficida® (10, 25, 50, 75, 100, 150, 200, 250, 300, and 400 mg/L) in a semi-static system over 96 h, with test solutions replaced every 24 h. Concentrations of Aficida® assessed throughout the study represent the nominal concentration of the formulated product. Negative (dechlorinated tap water, pH 7.6–8.3, hardness 250 mg CaCO_3 /L) and positive (13.7 mg Cr(VI)/L-treated) control fish [40] were run simultaneously with Aficida®-exposed fish. Each replicate consisted of ten organisms in a 1 L solution volume, and was performed in triplicate. Fish were not fed throughout the experiment. Lethality was the toxicity end-point. Fish were visually examined daily and considered dead either

when no respiratory movements were observed or there was no sudden swimming in response to gentle touching.

2.5. Genotoxicity assay

2.5.1. Micronucleus assay

Each experiment was conducted using five fish, following the same experimental design described in Section 2.4, with specimens exposed to four concentrations of test compound (50, 75, 100, and 157 mg Aficida®/L, representing 22, 33, 44, and 70% of the Aficida® LC-50_{96h}, respectively; see Section 3.1). Negative (dechlorinated tap water, pH 7.6–8.3, hardness 250 mg CaCO_3 /L) and positive (5 mg cyclophosphamide/L) controls were conducted and run simultaneously with Aficida®-exposed fish. The frequency of MN was determined on peripheral circulating blood erythrocytes, 48 and 96 h after initial treatment, as recommended [41]. Three independent experiments were performed and run simultaneously for each experimental point.

2.5.2. Analysis of micronuclei (MN)

At the end of each experiment, fish specimens were killed by severing the spinal column behind the opercula. Two drops of peripheral blood from the specimens were smeared onto precleaned slides. Afterwards, slides were air dried, fixed with 100% (v/v) cold methanol (4°C), and then stained with 5% Giemsa solution. Slides were coded and blind-scored by one researcher at 1000 \times magnification. The frequency of MN was determined by analyzing 1500 mature erythrocytes from each fish, as suggested [42,43], and is expressed as the total number of MN per 1000 cells. MN frequency was determined following previously reported examination criteria [43–45]. Briefly, the criteria employed for MN identification in intact cellular and nuclear membrane were as follows: diameter less than 1/3 of the main nuclear diameter; non-refractability; staining intensity similar to or lighter than that of the main nucleus; no connection or link with the main nucleus; no overlapping with the main nucleus; an MN boundary distinguishable from the main nuclear boundary [46].

2.6. Cytotoxicity assay

Frequencies of mature erythrocytes and erythroblasts were determined by blind scoring by one researcher at 1000 \times magnification, by analyzing a total of 1500 erythrocyte/erythroblast cells from each fish specimen in those slides employed for MN analysis, and expressed as the total number of erythrocytes and erythroblasts in 1000 cells.

2.7. Statistical analyses

Data of laboratory toxicity tests were analyzed using the Probit Analysis Program, version 1.5 (www.epa.gov). Analysis of variance (ANOVA), Mann-Whitney (Wilcoxon), and one-tailed Student's *t*-tests for independent samples were applied to compare data from the three independent experiments as mean values of MN/1000 erythrocytes and erythrocytes/1000 cells between treated and control groups employing the Statgraphics 5.1 Plus software. The relationship between frequency of erythrocytes/erythroblasts and MN data was evaluated by correlation analyses. The level of significance chosen was 0.05, unless indicated otherwise.

3. Results

3.1. Acute toxicity assays

HPLC results of chemical analyses showed no changes in the concentration of the toxicant in treatments during the 24 h interval replacements of the testing solutions.

Probit analysis of the mortality experiment allowed determination of the LC-50 of Aficida® after 24, 48, 72, and 96 h of exposure, with mean values of 344.3 (range, 320.2–379.8), 277.3 (range, 218.2–380.0), 243.6 (range, 229.1–258.5), and 225.5 (range, 210.5–240.6) mg/L Aficida®, respectively. Overall, regression analysis revealed that the LC-50 values decreased as a negative linear function of exposure time within the 0–96 h treatment period ($r = -0.96$, $P < 0.001$).

3.2. Genotoxicity and cytotoxicity assays

Fig. 1 summarizes the results of the analysis of Aficida®-induced MN in circulating erythrocytes of specimens of *C. decemmaculatus*. The frequency of MN in cyclophosphamide-exposed (positive control) fish was significantly increased compared to negative control values when the analysis was performed at 48 h ($P < 0.01$) or 96 h ($P < 0.05$).

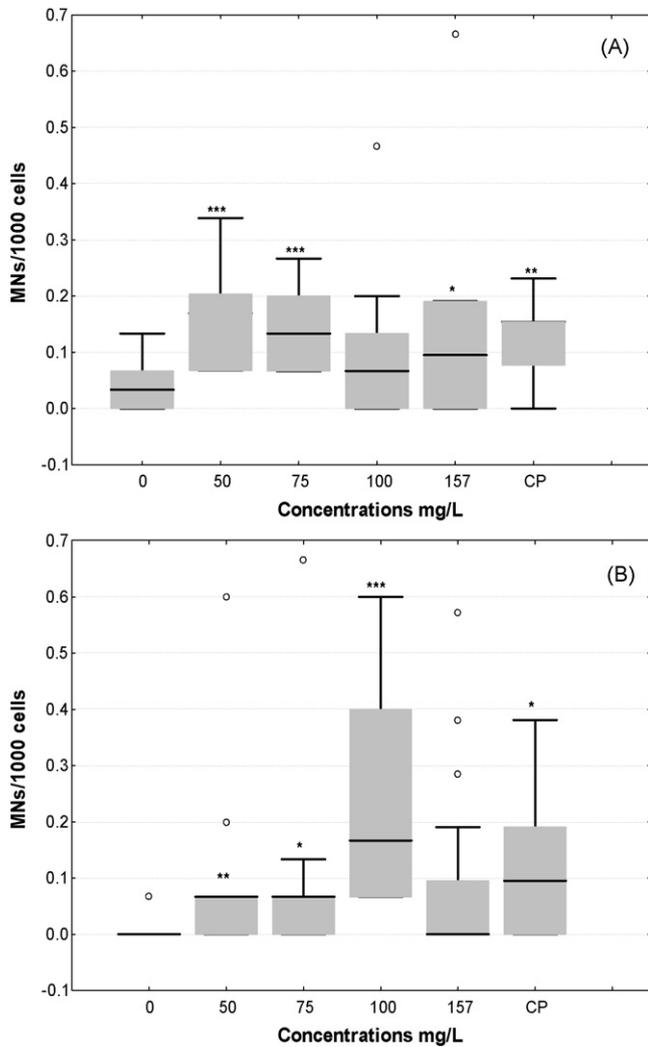


Fig. 1. Frequency of MN in circulating erythrocytes from negative controls and *Cnesterodon decemmaculatus* (Pisces, Poeciliidae) specimens exposed to different Aficida[®] concentrations. The frequency of MN was determined at 48 (A) and 96 h (B) after initial treatment by analyzing 1500 erythrocytes from each treated fish. The data are displayed as box plots, where the y-axis shows the range data. Each box encloses 50% of the data, with the median value of the variable displayed as a line. The top and the bottom of the box mark the limits $\pm 25\%$ of the variable population. The lines extending from the top and the bottom of each box mark the minimum and maximum values that fall within an acceptable range. Any value outside this range is displayed as an individual point (empty circles). Cyclophosphamide (5 mg/L) was used as positive control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

At 48 h, a significant increase in the MN frequency was observed in fish treated with Aficida[®] concentrations of 50 ($P < 0.05$), 75 ($P < 0.05$), or 157 ($P < 0.001$) mg/L, compared to negative control values. An increased MN frequency was observed in fish exposed to 100 mg/L, although not reaching statistical significance compared to negative control values. Overall, a regression analysis revealed that the frequency of MN values decreased as a positive linear function of the Aficida[®] concentration within the 0–48 h treatment period ($r = 0.76$, $P < 0.01$) (Fig. 1A).

In addition, Fig. 1B demonstrates that fish exposed for 96 h to all Aficida[®] concentrations but one (157 mg/L, $P > 0.05$) showed a significant increase in the frequency of MN compared to negative control values ($P < 0.01$, $P < 0.05$, and $P < 0.001$, for 50, 75, and 100 mg/L-treated fish, respectively). A regression analysis revealed that the frequency of MN values increased at all Aficida[®] concentrations, within the 0–96 h treatment period ($r = 0.51$, $P > 0.05$).

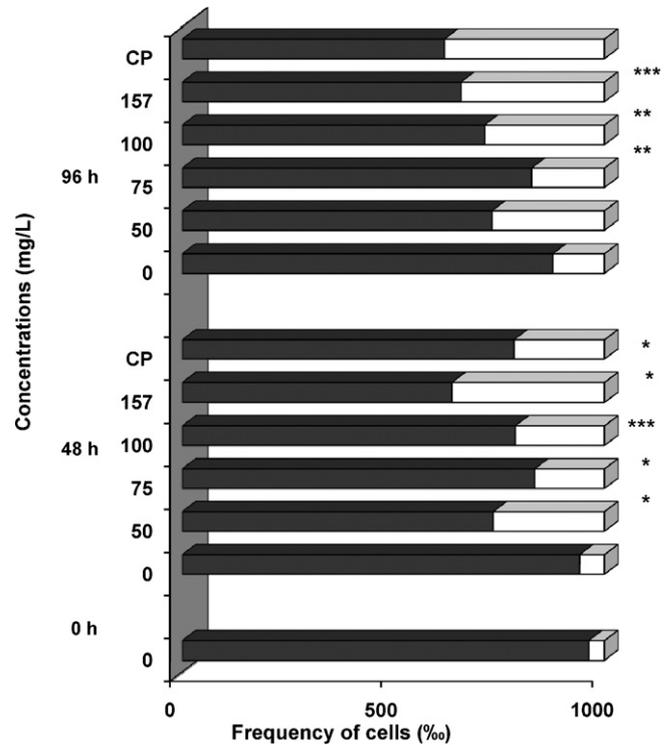


Fig. 2. Frequency of circulating erythrocytes (black bar areas) and erythroblasts (white bar areas) in circulating blood from negative and positive controls, and *Cnesterodon decemmaculatus* (Pisces, Poeciliidae) specimens exposed to different Aficida[®] concentrations. The frequency of erythrocytes and erythroblasts was determined at 48 and 96 h after initial treatment. The erythrocyte and erythroblast frequencies were determined by analyzing 1500 cells from each fish and are expressed as the total number of erythrocytes/erythroblasts in 1000 cells. Cyclophosphamide (5 mg/L) was used as positive control. ** $P < 0.01$; *** $P < 0.001$.

The results of the analysis of the proportion of circulating erythrocytes and erythroblasts in the blood of fish exposed for 48 or 96 h are shown in Fig. 2. When the erythrocyte:erythroblast ratios in the cyclophosphamide-exposed (positive control) fish were evaluated, a significant decrease and a concomitant increase in the frequencies of erythrocytes and erythroblasts, respectively, were observed in the blood of those positive control fish exposed for 48 ($P < 0.05$) and 96 h ($P < 0.001$), compared to negative controls. The results showed that, at 48 h treatment, a significant decrease and a concomitant increase in the frequencies of erythrocytes and erythroblasts, respectively, were observed within the 50–157 mg/L concentration range compared to negative controls ($P < 0.05$, $P < 0.001$, and $P < 0.05$, for 50–75, 100, and 157 mg/L-treated fish, respectively) (Fig. 2). When the analysis was performed after 96 h treatment, no alteration in the erythrocyte:erythroblast ratios were observed in 50- and 75-mg/L-treated fish. On the other hand, a significant decrease of erythrocytes and a concomitant increase in the frequency of erythroblasts were found when fish were exposed to either 100 or 157 mg/L ($P < 0.01$) (Fig. 2). Overall, a regression analysis revealed that the decreased frequency of erythrocytes, followed by the concomitant increase in the frequency of erythroblasts, varied as a positive function of the Aficida[®] concentration in fish treated for 48 ($r = 0.86$, $P < 0.05$) or 96 h ($r = 0.85$, $P < 0.05$) (Fig. 2).

4. Discussion

In the present report, the acute lethal toxicity, genotoxicity, and cytotoxicity of the pirimicarb-containing technical formulation insecticide Aficida[®] were evaluated on specimens from

C. decemmaculatus (Pisces, Poeciliidae) exposed under laboratory conditions. Regarding its acute lethal effects, the chemical can be classified as a low-concern agrochemical, according to the scoring used by the Office of Pollution Prevention and Toxics of the EPA [47,48].

Assessments of the genotoxic potential of pirimicarb, by a range of assays, have given inconclusive or contradictory results [24]. In spite of these discrepancies, pirimicarb has been classified as a moderately hazardous compound (class II) by WHO [6]. Our observations are in agreement with this classification.

Previous reports demonstrated the ability of both pure pirimicarb and its formulated product Aficida® to induce DNA single-strand breaks, sister chromatid exchanges, and structural chromosomal aberrations in mammalian cells *in vitro* [12,29]. Available information on the genotoxic property(ies) exerted by pirimicarb under *in vivo* conditions is scarce. Pilinskaia [31] reported an increase of chromosomal aberrations in occupationally exposed workers. Vera Candiotti et al. [23] recently demonstrated the ability of Aficida® to increase the frequency of micronucleated erythrocytes in *R. arenarum* tadpoles exposed under laboratory conditions.

Toxicity studies have been conducted on some metabolites of pirimicarb: three carbamate metabolites, three hydroxypyrimidine metabolites, and three guanidine metabolites. Two of the three carbamate metabolites, namely desmethyl pirimicarb and desmethylformamido pirimicarb, were found to be of the same order of toxicity as pirimicarb itself. Lower toxicity has been reported for the remaining seven derivatives [24]. Accordingly, it has been suggested that the genotoxicity of the pirimicarb may be related mostly to the effect of the desmethyl pirimicarb and/or the desmethylformamido pirimicarb metabolites [24]. The mechanisms by which *N,N*-dimethylcarbamate and their carbamate metabolites exert genotoxicity are not yet established. However, our current findings verify previous results describing the genotoxicity of pirimicarb through the induction of chromosomal damage and DNA single-strand breaks, both *in vivo* and *in vitro* [12,29,31]. Thus, the inconsistent results reported by different research groups regarding the deleterious effects of pirimicarb might be attributed to differing abilities to convert this pesticide into its carbamate derivatives by the various cellular systems employed [24,28,29,31]. The deleterious effect induced by the insecticide on vertebrate cells may be attributed either to the pesticide itself or to metabolites formed during the period of treatment.

Comparing the LC-50_{96h} values which we observed for *C. decemmaculatus* exposed to Aficida®, namely 225.5 mg/L (range, 210.5–240.6 mg/L), with those from the literature for several fish species in standardized toxicity tests, *C. decemmaculatus* is within the range between the most and least sensitive fishes, the rainbow trout *Oncorhynchus mykiss* (Salmoniformes, Salmonidae), LC-50_{96h} = 29–129 mg pirimicarb/L [20] and the common carp *C. carpio* (Cypriniformes, Cyprinidae), LC-50_{96h} = 410 mg pirimicarb/L [49], respectively. Much higher toxicity has been reported for the aquatic microcrustacean *D. magna* [50] and for the mosquito *C. quinquefasciatus* [19], with EC-50 = 0.014 mg/L and LC-50_{24h} = 8.5 mg/L, respectively. We have recently reported LC-50_{96h} = 181.7 mg/L Aficida® (50% pirimicarb) for tadpoles of *R. arenarum* following *in vivo* exposure [23]. Our current results in *C. decemmaculatus* agree well with these results and extend previous reports indicating that, depending upon the agrochemical, fishes may be more resistant than amphibians when acutely exposed to the same pesticide [51–53], although contrary results have also been reported [54,55].

Fish are appropriate aquatic vertebrates to be used as environmental genotoxicity bioindicator organisms, due both to their role in the aquatic trophic chain and their sensitivity to low concentrations of genotoxic substances, characteristic of polluted aquatic

environments [56]. Genotoxic effects on fish can be evaluated by several techniques, especially MN analysis in peripheral blood erythrocytes, a sensitive and reliable indicator of DNA damage. Micronuclei can originate both from acentric fragments resulting from chromosomal breaks which are not incorporated into the main nucleus and from whole chromosomes delayed during cellular division anaphase. These characteristics allow the detection of damage provoked both by clastogenic and aneugenic chemicals [57]. The piscine MN test has been extensively applied as an end-point for genotoxicity to assess the impact of pollution on contaminated areas (*in situ* assay) [56,58–62] as well as for screening different compounds after direct or indirect exposure (*in vivo* assay) [42–44,63–66]. Examples include MN induction in specimens from populations exposed to various anthropogenic activities, e.g., *Astyanax jacuhiensis* (Characiformes, Characidae) at petrochemical sites [58]; tilapias *Oreochromis niloticus* and *Tilapia rendalli* (Perciformes, Cichlidae), and common carp *C. carpio* (Cypriniformes, Cyprinidae) exposed to a complex mixture of organic pollutants from domestic sewers, industrial residues, agricultural and agroindustrial activities, as well as numerous inorganic substances of industrial sources [59,62]; and grey mullet *Mugil cephalus* (Mugiliformes, Mugilidae) for assessing genotoxic pollution in the marine environment [56]. Enhanced frequencies of micronucleated erythrocytes were observed under laboratory conditions in specimens of green snakehead *Channa punctatus* (Perciformes, Channidae) exposed to the herbicides pentachlorophenol and 2,4-dichlorophenoxyacetic acid, and the insecticides chlorpyrifos, cypermethrin, and malathion [63,66–68]; goldfish *Carassius auratus* (Cypriniformes, Cyprinidae) exposed to the herbicide glyphosate [43]; Nile tilapia *O. niloticus* (Perciformes, Cichlidae) exposed to the herbicide trifluralin [69]; and *Garra rufa* (Cypriniformes, Cyprinidae) treated with the insecticide lambda-cyhalothrin [70], among other species. In Argentina, in particular, reports in which the frequency of agrochemical-induced MN has been used as bioassay for detecting genetic damage induced in native vertebrate aquatic species, including amphibians, reptiles and fishes, are rare. While Lajmanovich et al. [71] demonstrated that exposure under laboratory conditions to the synthetic chlorinated insecticide-acaricide endosulfan increased the frequency of MN in *Hypsiboas pulchellus* tadpoles (Anura, Hylidae), an enhanced frequency of MN has also been observed in *Scinax nasicus* (Anura, Hylidae) tadpoles from ponds of agricultural landscapes at large-scale production with the herbicide glyphosate-resistant soybean as the dominant crop [72]. Cabagna et al. [73] reported the induction of MN under laboratory conditions by the pyrethroid insecticide cypermethrin in *Odonotophrynus americanus* (Anura, Cycloramphidae) tadpoles. Recently, we demonstrated an increased frequency of MN in erythrocytes from *R. arenarum* (Anura, Bufonidae) tadpoles exposed to Aficida® [23]. Finally, Poletta et al. [74] demonstrated the ability of the glyphosate-containing formulation herbicide Roundup® to induce MN in erythrocytes of broad-snouted caiman *Caiman latirostris* (Crocodilia, Alligatoridae) after *in ovo* exposure. An increased frequency of micronucleated erythrocytes was found in the Uruguay tetra *Cheirodon interruptus interruptus* (Characiformes, Characidae) after exposure to the insecticide lambda-cyhalothrin [75] or in specimens collected in ponds which flow through horticultural lands wherein great amounts of pesticides are applied [76]. Our present findings in *C. decemmaculatus* represent the first evidence of the acute toxic, genotoxic, and cytotoxic effects exerted by Aficida® under laboratory conditions obtained in another native Argentinean fish species.

Results from the analysis of Aficida®-induced MN in *C. decemmaculatus* were obtained after continuous treatments lasting up to 96 h. The analysis revealed, in spite of clear genotoxic properties exerted by the pesticide, some effects that can be related to

the *in vivo* exposure protocol that can lead to the underestimation of the damage quantified by the end-point employed, i.e., the MN frequency in peripheral mature erythrocytes. These included the induction of a selective cell loss by xenobiotic-induced cell death of the most damaged cells at an early exposure time, leaving only a reduced proportion of cells not severely damaged, and then able to persist in circulating blood; the inability of the most severely immature and damaged cells to enter mitosis, cells which are not included in further analysis; and the induction of a selective delay in the cell-cycle progression of the most severely immature and damaged cells delaying the presence of micronucleated erythrocytes in circulation; that is, the higher the damage level, the longer exposure lapse required for a micronucleated erythrocyte to be included in the analysis. Finally, piscine erythroblasts may be able to repair the damage induced by the pesticide.

Nuclear lesions might also be affected by other factors, namely erythropoiesis, required time for cellular maturation, and lifespan of erythroblasts and erythrocytes, as suggested by Udroui [41]. Thus, to achieve a further elucidation of Aficida[®]-induced damaging mechanism(s), the ratio of mature erythrocytes and erythroblasts circulating in those pesticide-exposed fish committed to MN analysis was analyzed. The results reveal a clear concentration-related alteration of the erythrocyte:erythroblast ratios in all Aficida[®]-treated fish. A significant decrease was observed in the frequency of mature erythrocytes with increasing concentration of the pesticide, regardless of the harvesting time. Our results suggest an alteration in the erythrocyte kinetics in pesticide-treated fish if we consider that MN-carrying erythrocytes tend to be removed faster from circulation than normal erythrocytes, due to one or more of the factors mentioned above. However, erythrocyte:erythroblast ratios can also reflect the balance between other biological factors, such as cell maturation rate, immature cell input, and cell removal by the spleen and liver [23]. Changes in population dynamics of several fishes species, often associated with pesticide pollution of agricultural areas or water reservoirs, is known worldwide. Other factors, e.g., over-exploitation, diseases, changes in reproductive patterns, and/or habitat loss, can also contribute to this situation. Negative effects against wild populations of mosquitofish *Gambusia holbrooki* (Cyprinodontiformes, Poeciliidae) [77], Northern bluefin tuna *Thunnus thynnus* (Perciformes, Scombridae) [78], North Pacific hake *Merluccius merluccius* (Gadiformes, Merlucciidae) [79], medaka *Oryzias latipes* (Beloniformes, Adrianichthyidae) [80], common sole *Solea solea* (Pleuronectiformes, Soleidae) [81], golden sailfin goodeid *Girardinichthys multiradiatus* (Cyprinodontiformes, Goodeidae) [82], white sturgeon *Acipenser transmontanus* (Acipenseriformes, Acipenseridae) [83], Nile tilapia *O. niloticus* (Perciformes, Cichlidae) [84], and brown trout *Salmo trutta* (Salmoniformes, Salmonidae) [85] exposed to dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethylene, multiple congeners of polychlorinated biphenyls, organochlorine, organotin, lindane, heptachlor, heptachlor epoxide, diazinon, aldrin, dieldrin, methyl parathion, fenthion, and DDT (including its metabolites DDE and DDD) pesticides, respectively, have been reported. Although environmental pollution might interfere with normal fish growth, development, and susceptibility to disease, the induction of genetic injury into DNA after chronic exposure to agrochemicals is, perhaps, the most relevant biological effect. Our results highlight that this native species provides a suitable and useful experimental model for biomonitoring aquatic ecosystems.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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